THE SYNTHESIS OF NUCLEIC ACID BY VIRUS-INFECTED BACTERIA[1]

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It is a great privilege to address the Society of American Bacteriologists, and I welcome the opportunity to tell you my thoughts concerning various aspects of the problem we have studied for the past few years, namely the biochemistry of virus synthesis. In the course of these studies, it became clear that the major problems were those of methodology and of the theory of modern biology, and not merely the collection of data. Many of these questions were approached in a social way, by intensive discussion with my many colleagues in many places and in many disciplines. For their cooperation and assistance, I wish to express my indebtedness.

It has been my experience, not at all damaging, to be considered a bacteriologist by many biochemists and a biochemist by many bacteriologists. The pleasure of being deemed both by the Society, without detriment to the disciplines of either, leads me to recall to you the remarks of the great biochemist, Sir Frederick Gowland Hopkins, who asserted that in exploring and cultivating the fields of nature the chemists were best provided with the machinery for this cultivation, but that the biologists knew best the lay of the land. This guiding principle of the usefulness of several disciplines has been well exemplified in the recent advances around the bacteriophage problem. There are few fields of biology today which have been so explored and cultivated by the independent but combined attacks of bacteriologists, physicists, chemists, geneticists, etc. Furthermore, it is safe to say that in this field no man can stand alone. For although the many noteworthy contributions may have come from individuals, they arose in a framework of socially derived knowledge and an extremely critical evaluation by groups and individuals of initiating hypotheses, of the experimental designs and data, and certainly of the conclusions therefrom obtained. Indeed, concerning the social character of this critical evaluation, a special point must be made. In the bacteriophage field, criticism has been elevated to a method of work of considerable importance. The various aspects of critical evaluation have been organized by the workers themselves, and I believe it to be true that this organized criticism, expressed frequently among ourselves in the sharpest terms, has considerably increased the productivity of the individual workers and the value of their contributions.

It would be a mistake, however, to imagine that rubbing shoulders with a variety of disciplines will necessarily affect certain more deepseated notions con-

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cerning the nature of a problem and an experimental approach to it. Until recently, in the field of virology, the role of the biochemist was affected by a distinct genetic conception of the nature of a virus. This is exemplified even now by the continued use of the expression, "self-duplicating". Modern workers in genetics will agree that it is not known that a gene synthesizes itself, but that the process of genetic and chromosomal duplication must be a complex process involving diverse interactions between the substance to be duplicated, the enzymatic equipment of the cell, and other substances in the environment. Nevertheless, the term "self-duplicating" continues in use, in genetics and virology despite the mechanisms of synthesis implicit in it. The term is often used in serious writing today, but qualified in an additional paragraph. Since the problem of the interaction of the virus with its environment was minimized by the tacit adoption of the concept of self-duplication, the most important problem in virus reproduction became the problem of the nature of the virus itself. Indeed, until World War II, this was the focal point of study, and this type of study was intensified during the war by the need for more potent vaccines.

Although it is quite important to make vaccines, and to understand the properties of isolated viruses in order to obtain clues as to what is happening in virus synthesis, the following considerations led me to leave this kind of approach. All the data of chemical virology strongly indicated the absence in most viruses of numerous coenzymes and enzymes considered to be important in biological systems for the production of energy and the transformation of matter. It followed that since the host had the necessary enzymes for these essential changes, one might expect to find the host playing a very important role in virus synthesis. This is to do no more than rephrase the usual definition of virus, i.e., a minute organism capable of multiplication only within living cells. Furthermore, it may be recalled that by the end of 1945, it had become abundantly clear that substances such as the sulfa drugs and penicillin were ineffective in combating the primary effects of virus infection. We might imagine that if penicillin had worked in various virus infections that the nature of virus synthesis would not be pursued quite so strenuously. The time seemed ripe for a biochemical study of phenomena appearing during those interactions of a virus and its host leading to the production of more virus. This approach may be considered new because despite some studies on the effect of changes in the nutritional state of the host on virus infection, at the time these studies were begun there were practically no data on the metabolism of virus-infected cells. This approach was not theoretically inacceptable since it envisaged understanding the separate properties of virus and host.

Studies were begun on the metabolism of *Escherichia coli* infected with one of a group of T bacterial viruses, since the biological criteria of establishing and estimating infection and virus synthesis had been worked out for this system. For the details of the phage methods and other attributes of the system, I shall refer you to various reviews on the subject, or even better to the intensive course on bacteriophage given each summer at the Long Island Biological Laboratory at Cold Spring Harbor.

There are three major phases of virus infection in this system, namely adsorption and penetration, synthesis of virus components, and lysis and liberation of virus. We shall limit our discussion in the main to the second phase, that of the synthesis of virus components, and particularly that of virus nucleic acid.

The nature of these components are seen from an examination of the viruses. We have been concerned mainly with three tadpole shaped phages, T_2, T_4, and T_6, which contain nucleoprotein organized within the head, which is bounded by a distinct membrane. These phages are serologically related but are readily distinguished by their host ranges, plaque form and size, adsorption and other properties. The T_4 and T_6 strains we have used require an adsorption cofactor, such as tryptophan (9). It has been shown that the isolated infectious viruses contain two major constituents, protein and desoxyribose nucleic acid (DNA), the latter amounting to about 40% of the dry weight of the particle (1). There are occasional reports of the presence of very small amounts of other substances, ribose nucleic acid, carbohydrate, and lipid. In the majority of cases where varying amounts of RNA were found, the method of virus preparation facilitated contamination of the preparation with host debris which probably was present. We have applied immunochemical procedures in order to analyze the extent of contamination with host debris and to assist in virus purification (8). After such rigorous procedures, the demonstration of RNA in virus has not been accomplished.

Another difficult structural problem arises around the nature and origin of the nucleic acid coating of virus particles produced in a host deficient in active autolytic systems, especially desoxyribonuclease. It is agreed in numerous laboratories that these viruses when produced in bacteria grown in a lactate medium are less stable when isolated, than the same virus isolated from a lysate of bacteria, prepared in a broth or glucose medium. We have shown that unstable preparations of the former appear to have a coating of DNA sedimentable with the virus, reactive with streptomycin, and removable by desoxyribonuclease (3). These experiments in which the formation of streptomycin nucleates, dissociable by salt, was first described, have given rise to one of the numerous theories of the mode of action of streptomycin, namely an action based on its reactivity with nucleic acid. Now, in so far as removal of the DNA coating appeared to increase virus stability without loss of activity, and capsular materials of bacteria, rickettsiae (40) and possibly some viruses such as vaccinia (18), are not an uncommon phenomenon, this coating could be thought of as a special product of normal virus synthesis. However, since virus instability reveals itself in the leakage of nucleic acid from the head of the tadpole shaped particles, it is possible that the coating of nucleic acid on intact particles is due to adsorption of nucleic acid derived from damaged particles. In short, it is difficult to decide whether the DNA coating of these unstable virus preparations is a cause or effect of virus instability, or both. In any case, our experiments of the past few years have attempted to avoid, rather than solve this problem, by using media from which stable uncoated virus may be isolated.

From such virus particles resistant to proteolytic enzymes and desoxyribonu-

clease, polymeric DNA free of protein has been isolated in high yield by a procedure involving disruption of the virus with urea (2). This isolation was developed in order to attempt the transformation of hereditary characteristics of phages with DNA. Our experiments in this direction were unsuccessful and as we shall see, the evidence that DNA plays a genetic role in this system, while considerable and suggestive, is at best indirect. It may be noted that the cytosine content of the DNA of the phages appears to be quite low, in contrast to some other nucleic acids, such as mammalian DNA or the DNA of *E. coli*.

It has been reported that T_4 is inactivated less readily by ultraviolet radiation than are T_2 and T_6 (30). We have observed the most active preparations of T_4 contain proportionately less DNA per active particle than the most active preparations of T_2 and T_6 (8). It has been observed that a plot of the efficiency of inactivation as a function of the wave length of ultraviolet irradiation strongly resembles a nucleoprotein absorption spectrum (42). From experiments of Luria, Williams, *et al.*, T_4 gives a lower absorption per particle at 2600 Å than does T_2 in experiments in which the absolute numbers of all virus particles, active and inactive, were counted (33).

The fact that practically all of the P of these viruses is to be found in a single nucleic acid, of the desoxyribose type, distinguishes these organisms from all cells which have been examined with respect to their nucleic acid content and have been found to contain both RNA and DNA. Although some few cells are known in which the DNA content exceeds that of RNA, in the majority of growing dividing bacteria studied, RNA greatly exceeds DNA. In our host, *E. coli*, the ratio of RNA:DNA may be of the order of 3–5:1, depending on the character of the medium used for growth, and as in other cells, the DNA is associated with apparently nuclear bodies, whose structural qualities may be followed after appropriate Feulgen staining (32).

In the basic metabolic experiment, bacteria were mixed with purified virus in media of known composition under defined environmental conditions in such a fashion that each bacterium adsorbed at least one virus particle. The host cells, each weighing about 10^{-12} g, were actively growing and assimilating carbon, nitrogen and phosphorus compounds. Their enzymes were reproducing all of the components of the cell, including RNA and DNA. The virus particles contain about 1/1000 the mass of the host, appear essentially inert, and have not been shown to contain any enzymes involved in the development of energy or the synthesis of any compound, although tested in numerous reactions. The effect of infection of the cell by even one of the minute parasites is catastrophic.

At the onset, we showed that T_2 infected cells stop multiplying (1). It is curious to note that this inhibition had not been looked for previously under conditions of virus synthesis, although it had been shown that ultraviolet-inactivated T_2 inhibited multiplication of the bacterium (29). On infection with either active or inactive virus, cytological studies by several workers (17, 32) indicate that the Feulgen-positive nuclear bodies of the cell are disrupted and the Feulgen-positive material is scattered to the periphery of the cell, although the cell itself is still intact. That this limited controlled disintegration of host

structure after initial infection does not go on to complete autolysis is in part a function of the continuing energy supply to the cell. It has been shown that inhibition of respiration with cyanide, iodoacetate or even the lack of O_2 at the moment of infection with T_2r^+ under certain conditions results in internal enzymatic anarchy culminating in complete autolysis (7).

The study of the respiration of infected bacteria revealed no change in the rate of O_2 consumed or in the R.Q. (1). This indicated an inhibition of the synthesis of respiratory enzymes, although assimilation of carbon, nitrogen and phosphorus continued quite vigorously. Not only does the infected cell stop multiplying, but Monod and Wollman have shown that the infected cell is unable to adapt to use certain new substrates (34). The cell has been reconverted for virus synthesis with a minimum of retooling; new products will now appear.

Protein synthesis continued without a stop from the moment of infection (5). It is not known whether this is all virus protein, although it is clear that most of the protein produced is virus protein. We have shown in numerous experiments that the requirement for virus protein constituents, such as tryptophan, determining the ability to produce virus, is manifested throughout the latent period. Furthermore, these requirements and virus synthesis may be interfered with by appropriate analogs (4, 21). The rate of utilization of an essential protein constituent, such as tryptophan, is a function of the particular virus used to infect the cell, thereby indicating marked differences in the tryptophan contents of T_2, T_4, and T_6 (38).

After infection, the ultraviolet absorption of the cells at 2600 Å increased, indicating a continuing purine and pyrimidine synthesis (10). The magnitude of this increase corresponded closely to the amount of nucleic acid which began to appear a few minutes later. These increments examined over the ultraviolet range of 2300 to 3200 Å revealed a typical nucleoprotein spectrum.

The synthesis of protein and of the nucleic acid bases was followed after a significant lag of 7 to 10 minutes by the synthesis of polymeric DNA (2). This lag in DNA synthesis was significantly decreased by increasing the multiplicity of infection (10), a ratio of virus to cell of about six producing the minimal lag before DNA synthesis began. The redistribution of the bacterial nuclear fragments appears to be completed before DNA synthesis starts.

Other functions seem to be related to the beginning of DNA synthesis. Changes have been observed in the susceptibility of the infected cell to temperature and urethane at this point. Study of the inactivation by irradiation of intracellular units capable of becoming virus suggests that some type of multiplication begins at about this time (26). The induction of virus mutation by irradiation of the infected cell has been reported only during a period similar to that of DNA synthesis (27).

From the time of adsorption of the virus to the cell until several minutes after an increment in DNA appears, disruption of the cell does not liberate infectious virus particles, not even the original infecting virus. As Doermann has found (19), intact intracellular virus appears at about the half-way mark in the latent

period, or as I have shown in collaboration with Doermann, several minutes after DNA synthesis begins (7). Furthermore, the rate of this formation of intracellular virus is that of DNA synthesis, which not only appears to determine the inception but also the amount of virus synthesis, since as we have shown, the amount of virus produced per cell approximates the amount of DNA observed to be produced in that cell (9).

Estimations of the increments in protein, ultraviolet absorption at 2600 Å, DNA, and intracellular virus show that the rates of synthesis are essentially constant as in figure 1. They are independent of the amounts of virus known to be formed within the cell, whether these be 4 virus particles or 100. The rates of DNA synthesis for instance are independent of the r character of the virus or

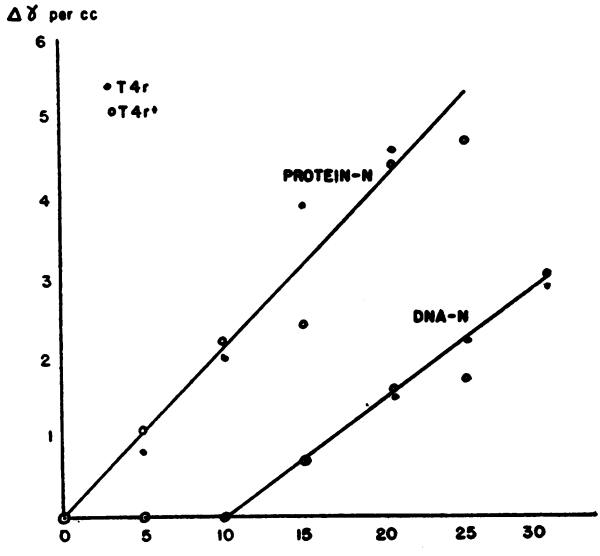

FIG. 1. A comparison of protein and DNA synthesis in cells multiply infected with T_4r^+ or T_4r virus. Abscissa: minutes.

whether the virus is T_2, T_4, or T_6 (9). Further the amounts of substance synthesized are of the order of that produced by the bacterium, rather than that of a particle one-thousandth of the cell's mass. We have concluded from these considerations that the production of virus constituents is a function of some preformed constant element in the system. Making this slightly more daring, we have concluded that the virus polymers are synthesized in stages by the host enzymes according to the specificities imposed by the infecting virus particles, i.e., the host synthesizes the virus (2). These conclusions are not yet subject to direct proof and I stress only that the conclusions are reasonable and do not contradict any data so far obtained on the system.

Now infection produces about a four-fold stimulation of DNA synthesis, concomitant with a total inhibition of RNA synthesis, as represented in figure 2. This has been represented as a shunt in P utilization, i.e., the P assimilated,

which would become incorporated into RNA under conditions of growth, is now shunted completely into DNA synthesis (2).

At the time that this work was begun, the theories of nucleic acid synthesis leaned most strongly to the view that there was a conversion of RNA nucleotides to DNA. It was shown in contradiction of this hypothesis that RNA was inert in this virus system: first, that the pentose of RNA did not change in amount (5), and secondly, that in the incorporation of P^{32} from the medium into virus DNA in infected cells significant amounts of radioactivity did not appear in the RNA (6). P did not appear to pass through a significant fraction of RNA nucleotides before being incorporated into DNA. That the bases of the RNA are equally inert in the infected cell seems likely, but has not yet been proved experimentally.

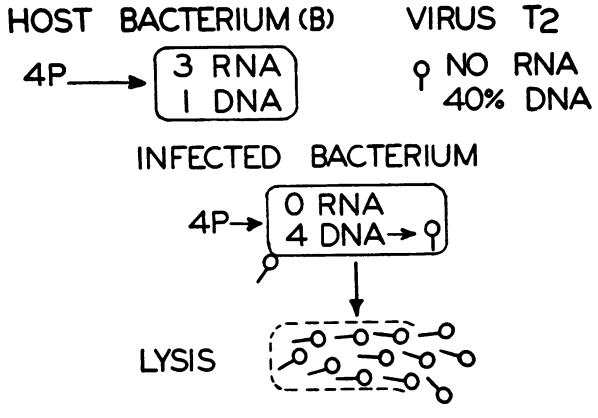

FIG. 2. The shunt in P utilization and nucleic acid synthesis during virus multiplication.

It was also shown by means of various isotope experiments with T_2r^+ and T_4r^+ (6), and later extended to T_6r^+ (35), that 70 to 80 per cent of the P used to make virus was derived from the medium after infection. It has been the important contribution of the group at the University of Chicago that they have striven most energetically to track down the source of the contribution of host P. In various isotope studies, they have presented evidence strongly suggesting that the host DNA is a major source of P, N and purine to virus synthesis (35, 25, 24). This fact must be noted in the light of the cytochemically observed disintegration of the nuclear bodies of the host bacterium.

In collaboration with Dr. Lawrence Weed, we have confirmed that host nucleic acid constituents are used for virus synthesis (41). We have grown the bacteria in the presence of a pyrimidine precursor, C^{14} labelled orotic acid, and have found the isotope exclusively in the pyrimidines of RNA, i.e., uracil and cytosine, and of DNA, i.e., thymine and cytosine, which were isolated as the pyrimidine nucleotides. On infection of washed labelled cells in a synthetic medium with either T_6r^+ or T_6r, the virus liberated on lysis was found to contain considerable amounts of the labelled pyrimidines derived from the host.

Under the conditions of our experiment, the patterns of DNA synthesis for T_6r^+ and T_6r are given in figure 3. Synthesis in T_6r infected cells stops earlier because of earlier lysis; in r^+ infected cells, lysis does not occur until several hours after synthesis stops. Two hypotheses may account for the transfer of host DNA to virus: *a*, that the host DNA plays a special role, perhaps a genetic role, by virtue of a specific fragment which is incorporated into virus, or *b*, that the host DNA is degraded to form low molecular materials utilizable early in virus synthesis, in a manner comparable to the utilization of other newly synthesized components, such as nucleosides and nucleotides. From the former hypothesis we might expect the amount of host component in T_6r^+ to equal that in T_6r, or if we could obtain early intracellular T_6r^+ that it be labelled to the

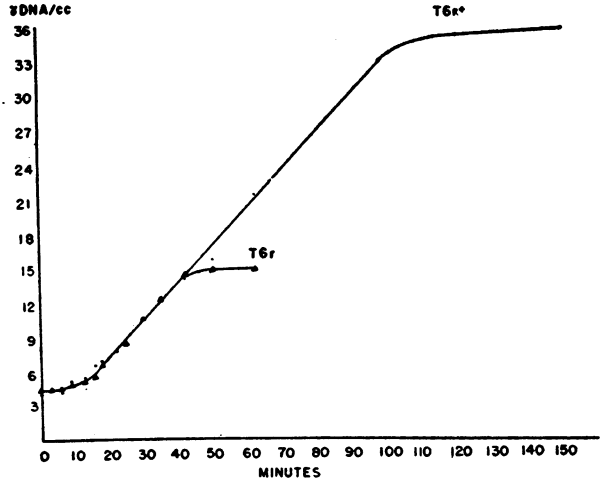

FIG. 3. A comparison of the synthesis of DNA in cells multiply infected with T_6r^+ or T_6r virus.

same extent as the final yield of T_6r^+. From the latter hypothesis we might expect the degree of incorporation of host components to be inversely related to the amount of DNA synthesis in the system. The data support the second hypothesis.

Not only have we compared a final yield of T_6r^+ with T_6r grown in identically labelled cells but also the final T_6r^+ with early T_6r^+. Early T_6r^+ was obtained after premature lysis of the same infected cells with 0.01 M NaCN. The major point which I wish to stress from the data of table 1 is that the relative amount of either host desoxycytidylic acid or host thymidylic acid in the viruses is inversely related to the extent of DNA synthesis in the infected cell. For example, the ratio of DNA synthesis in early T_6r^+ to late T_6r^+ was 1:4.3. The ratio of host thymine in early T_6r^+ to late T_6r^+ was 4.1:1. Since early T_6r^+ had obtained the maximal amount of host thymine, all T_6r^+ synthesized after this time, i.e., the last three quarters of virus synthesized, received no contribution from host pyrimidine. There was also to be observed a discrepant transfer of the

two host pyrimidines; other data suggest a degradation of host DNA to at least the level of nucleosides prior to resynthesis to virus DNA. From evidence of this type we have concluded that host DNA does not play a specific genetic role in virus synthesis.

These observations provide an explanation for certain results in which host nucleic acid provides essentially all of the substance necessary for virus DNA synthesis. This appeared to be the case for T_2 synthesis when very small amounts of virus were produced (22), or T_2 synthesis in the absence of externally supplied P (21) or for T_7 where the latent period of virus synthesis is exceedingly short (20). They reflect the availability of host DNA for virus DNA synthesis under conditions in which new DNA synthesis is not possible or necessary.

TABLE 1

The transfer of host pyrimidines to virus DNA

SUBSTANCE	BACTERIAL DNA	T6r+	T6r+	T6r
		Normal Lysis	Cyanide Induced Lysis	Normal Lysis
Increment in DNA mg/5 ml culture		0.208	0.048	0.075
Thymidylic acid counts/min/mg base	2780	217	894	766
Per cent of base derived from host		7.8	32	28
Desoxycytidylic acid counts/min/mg base	3420	401	1490	1252
Per cent of base derived from host		11.7	43	37

Does virus DNA play a specific genetic role in virus synthesis? Although it has not yet been possible to obtain direct functional evidence of DNA specificity as in pneumococcal transformations (2), other phenomena in the phage system warrant exploration from this point of view. The discovery of the genetic recombination of these phages, determined under conditions of full activity of the virus particles or of partial damage by radiation has raised the problem of a division of labor within the phage particle with respect to the determination of hereditary properties. By analogy to the pneumococcal transformation data, it appeared possible that specific DNA determined certain genetic properties.

It must be noted that the exchange of viral substance is a real phenomenon. On infection, the virus particles break down with extensive degradation of viral DNA (36, 28). The first generation of viral progeny then contain only a small portion of the DNA components of the original infecting particles, about 15 to 30%, depending on the conditions of the experiment. Lest we suspect that this limited transfer of these transferred virus DNA components represents an irreducible genetic core, I am informed by Dr. A. D. Hershey that in the next generation, there is transfer by the same proportion of this original DNA, thus further reducing this original material in the progeny.

Thus, in a manner completely unlike reproduction of bacteria by binary fission but in a fashion still consistent with our notion of the direction of host enzymes by portions of the infecting virus, the virus particle breaks apart as a prerequisite

for duplication, since it can in no other way provide its internal parts to affect the host's enzymes.

Now, genetic theory has stimulated a number of proposals, one of which we have tested. Our results in this are also pertinent to the mechanism of the interference phenomenon. The premise of a genetic division of labor based on the recombination data has been extrapolated to the notion of the complete independence of genetic units with respect to their duplication. This idea was especially advanced to explain the system first described by Luria (30, 31), in which he found that infection of the cell by several particles partially damaged by ultraviolet irradiation permitted virus synthesis, although each damaged particle alone was incapable of inducing reproduction. Since a correlation has been shown between radiosensitivity and the amount of DNA in these phages, as well as between radiosensitivity and nucleic acid absorption, the following assumptions were made: *a.* That separate units of nucleic acid absorb the radiation and are changed by it. *b.* That these damaged DNA units are capable of blocking the synthesis of the original undamaged DNA units.

From these assumptions, on infection of the cell by damaged particles, we might expect one of two results: *a.* A partial synthesis of DNA corresponding only to the undamaged units, if the synthesis and genetic duplication involved independent genetic units or *b*, a complete inhibition of DNA synthesis, if it was necessary for all determinants of the phage to be acting together. The latter result was obtained. There was a complete inhibition of DNA synthesis, as in figure 4. In these experiments, conditions were arranged to facilitate mutual or multiplicity reactivation, which accounts for the eventual beginning of DNA synthesis in the inhibited system. This delay in DNA synthesis was also shown to coincide approximately with the delay in appearance of intracellular virus. Therefore, we have concluded either that the reproduction of genetic determinants requires the coordinated activity of all the undamaged genetic units, or that an increase in DNA has nothing to do with the reproduction of genetic determinants (10).

Despite the delay in DNA and virus synthesis in cells infected with ultraviolet irradiated virus, synthesis of ultraviolet absorbing components continued as in the uninhibited infected cell. These have a nucleoprotein spectrum. Furthermore, we have observed only a slight inhibition in protein synthesis under these conditions despite the fact that no protein bound nucleic acid, RNA or DNA, is being synthesized in this system until reactivation. This phenomenon therefore accentuates the time relationships of protein, DNA, and virus synthesis observed in conditions of normal T_2r^+ synthesis, and contradicts the hypothesis of the mutual interdependence of protein and nucleic acid synthesis, at least in this system.

Further experiments have revealed that P assimilation in the cell occurs in the period of inhibition of DNA synthesis (15). Inorganic P was converted to organic P which accumulated in the acid-soluble fraction of the cell. Thus, treatment of the cell with T_2r^+ which had been inactivated by absorbing energy presumably in the DNA, specifically inhibited DNA synthesis, without appearing

to affect the synthesis of ultraviolet absorbing components, proteins, and P assimilation. We might imagine that a virus DNA fragment had been converted by radiation into a DNA analog which competed with normal DNA at enzyme sites which produced DNA polymers.

A problem which we consider crucial to our understanding of the details of the parasitic process is that of the P shunt. We consider that this single phenomenon of the P shunt manifested in part by the inhibition of RNA synthesis could result in the total deviation of synthesis of host components to that of virus constituents. The integrated structure of the cell contains RNA inti-

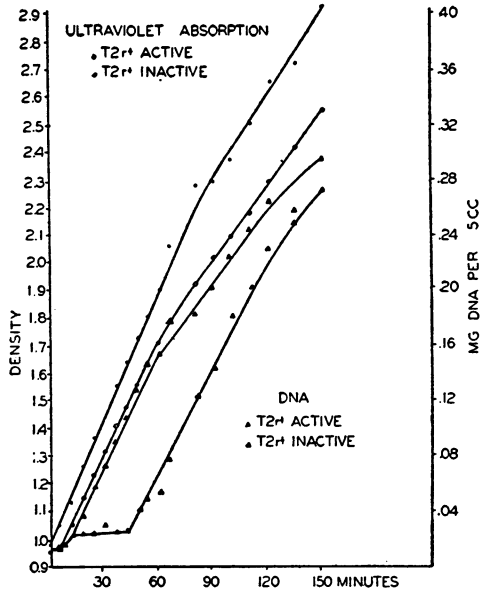

FIG. 4. A comparison of DNA synthesis and ultraviolet absorption increments at 2600 Å in cells infected with active or irradiated T_2r^+ (virus:cell = 6.0).

mately organized within it. It appears quite reasonable to imagine that the inhibition of RNA synthesis could stop the synthesis of all other materials normally closely associated with RNA, and dependent on the contiguity of RNA for the continuing elaboration of the cellular fabrics.

We began work several years ago in an effort to detect the pivotal point of the P shunt. Since most of the cell P is bound to ribose and desoxyribose, the problem is that of determining the origin of ribose and desoxyribose phosphates. We have recently demonstrated an enzymatic pathway for the formation of ribose-5-phosphate from 6-phosphogluconate (11) and Racker has succeeded in doing this for desoxyribose-5-phosphate (37).

The oxidation of 6-phosphogluconate by cell free enzyme systems from yeast was discovered in the 1930's but neglected for a decade. In collaboration with Dr. D. B. McNair Scott, we reinvestigated this yeast system and have now

extended our studies to extracts of *E. coli*, as well as to the intact bacteria. 6-Phosphogluconate is an enzymatic or chemical oxidation product of glucose-6-phosphate, the primary phosphorylated product of glucose. For the past decade, it has been considered that the single major route of degradation of glucose-6-phosphate in most cells and tissues is via the Embden-Meyerhof scheme, a pathway not involving 6-phosphogluconate nor giving any clue to the mode of formation of ribose.

In the study of the products of phosphogluconate degradation we have developed a series of new separation and microanalytical techniques. In addition to the characterization of products by several different chromatographic procedures, one microbiological method has proved of great value. We have used pairs of adapted and non-adapted bacteria in the specific fermentative analysis of such

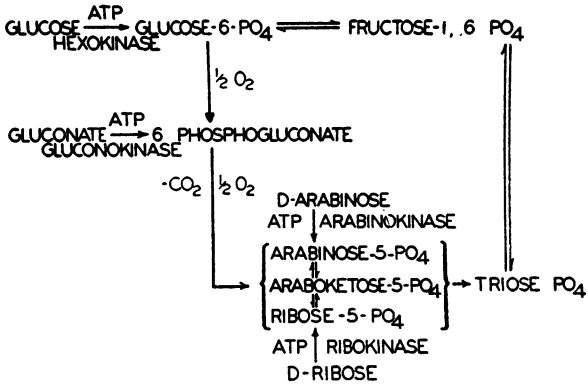

FIG. 5. The pathways of conversion of glucose and gluconate to triose phosphate

carbohydrates as gluconate, D-arabinose, and ribose (12). Furthermore, in each case we have shown that the adaptive enzyme making possible the metabolism of the specific substrate is a specific transphosphorylase, which with ATP forms the phosphorylated sugar as the metabolic intermediate (13, 14).

Briefly we have shown that ribose-5-phosphate is generated via the oxidative decarboxylation of phosphogluconate, the major isolable product of decarboxylation being a hitherto uncharacterized pentose phosphate. In yeast systems we have evidence to indicate that this compound has properties of a 1–2 enediol pentose phosphate. Araboketose-5-phosphate has been designated by Horecker and Smyrniotis as the primary product (23). These workers have confirmed our finding of the production of ribose-5-phosphate in this system. In *E. coli* systems, we have observed both the enediol in smaller amounts and large amounts of a compound apparently similar to araboketose. In addition, in both systems we have demonstrated arabinose-5-phosphate. Therefore, the relatively crude enzyme systems we have isolated from yeast and *E. coli* produce an equilibrium mixture of pentose phosphates, one component of which is ribose-5-phosphate.

It has also been shown that enzymes exist in *E. coli* for the conversion of ribose-5-phosphate to triose phosphate, another meeting point with the Emb-

den-Meyerhof scheme. Finally, according to Racker, this triose phosphate may be used with the appropriate enzyme of *E. coli* and acetaldehyde to form desoxyribose-5-phosphate (37).

This new oxidative pathway of carbohydrate metabolism is shown in figure 5. As has been mentioned, the phosphorylation of gluconate, D-arabinose, and D-ribose permits the insertion of these substrates into the oxidative pathway directly. Since we have recently proved that the oxidation of glucose-6-phosphate to 6-phosphogluconate is essentially irreversible in the intact growing or virus-infected cell, it is evident that the utilization of gluconate, for instance, in *E. coli* proceeds only via the oxidative pathway. We have observed that whereas this substrate is almost as efficacious as glucose for growth, it is a poor substrate for DNA and virus synthesis. That is to say, with gluconate and the sole use of the oxidative pathway, the usual excess of RNA over DNA may be produced readily for growth. However the cell is unable to maintain a stimulated DNA synthesis for virus synthesis through this same pathway.

In order to determine the actual quantitative relations of the pathways by which glucose is used in growth or virus synthesis, let us examine the method used to establish the irreversibility of the oxidative step leading to 6-phosphogluconate (14, 16). If we were to trace glucose utilization by each pathway, carbon by carbon, it would be evident that the oxidative pathway involves a complete loss of C_1 as CO_2 in the decarboxylation of 6-phosphogluconate to pentose-5-phosphate. C_1 in the Embden-Meyerhof scheme is fated to become the CH_3 group of pyruvate and by known metabolic schemes tends to be conserved or at least used to no greater extent than the rest of the pyruvate-carbon. In the metabolism of C_1-labelled gluconate in growth, virus infection, or merely oxidation, the appearance of C_1 as CO_2 approaches 100%, although half or less of the gluconate carbon appears as CO_2.

In contrast to this apparent exclusive use of the oxidative pathway in gluconate utilization under a variety of conditions, a different situation prevails with glucose utilization. As can be seen in table 2, both growing and virus infected cells liberate about 1.4 moles of CO_2 per mole of glucose or 23% of the total C appears as CO_2. If glucose were metabolized by the Meyerhof scheme, we might anticipate by known mechanisms a maximum of 23% of the total C_1 of the glucose metabolized or a minimum of O. Under conditions of growth on C_1-labelled glucose, we found 37% of the C_1 in the CO_2, a value considerably in excess of the maximum value permitted via the classical Meyerhof scheme, albeit far less than that observed in gluconate utilization. Therefore, it is concluded that both paths are in operation in growth on glucose, the oxidative pathway accounting maximally for 37% or minimally 37 minus 23%, i.e., 14% of the glucose utilization. The utilization of the remainder of the glucose is probably a function of the Meyerhof pathway.

In virus-infected cells, glucose utilization via the oxidative pathway is curtailed sharply. Markedly smaller amounts of C_1 appear as CO_2, although the total CO_2 production is unchanged. The apparently unchanged respiration on virus infection noted earlier hides within it a shift in the intramolecular degrada-

tion of the glucose, a shift impossible with gluconate. Whereas fairly considerable amounts of glucose as hexose phosphate were metabolized via the oxidative pathway in the growing uninfected cell, significant amounts of this hexose phosphate have now been shunted away into another pathway which does not involve ribose-5-phosphate as an intermediate.

Thus, we feel we may have come a little closer to the mechanism of parasitism in virus infection. It is suggested that in growth on glucose the ribose phosphate of RNA comes from the oxidative pathway, while in virus synthesis on glucose the desoxyribose phosphate of DNA comes from the Meyerhof scheme. It seems likely that the P shunt observed in virus infection occurs at the point of metabolism of glucose-6-phosphate. The mechanism of the determination of the route to be taken is still unknown.

TABLE 2

C_1 recovery in CO_2 produced during glucose utilization by E. coli

PHYSIOLOGICAL CONDITION	MOLES CO_2 / MOL GLUCOSE	$BaCO_3$ CTS/MIN/MG C	RECOVERY OF C_1 IN CO_2*	THEORETICAL C_1 RECOVERY	EXCESS
			Per Cent	*Per Cent*	*Per Cent*
Oxidation, no growth	3.22	212	54.4	53.7	0.7
Growth	1.32	156	37.7	22.0	15.7
T_2 Synthesis	1.38	121	29.1	23.0	6.1

* Calculated after correction for dilution.

In a very few years, many workers and especially my colleagues at The Children's Hospital of Philadelphia have demonstrated the biological similarities of other virus systems to the phage systems. In some respects, they have even outstripped the phage work. Just as an intensive chemical and biological study of the phage problem has produced many methods of preventing phage multiplication, we may anticipate that similar studies of virus multiplication in mammalian virus systems will result similarly in the discovery of controls of virus multiplication. A serious problem arises, however. Will it be possible to effect similar studies of virus multiplication in mammalian virus systems and subsequently extend them to man? The technical and financial difficulties that arise in biological and biochemical work with higher organisms are the key problems in extending the phage work to other systems; these difficulties can only be described as enormous. It would appear that the rapid extrapolation of the phage work to the virology of man is not really a problem of basic science, but rather one of scientific and social organization. In my opinion, the development of a rational chemotherapy of the virus infections of man will involve primarily the solution of this problem, and this is not only a matter for scientists. It is conceivable that industry will tire of its empirical approach to virus chemotherapy and apply its organized strength to that of a rational chemotherapy. Or even that some private foundation will attempt a more organized attack. In any case, we can note that in understanding the basic parasitic nature of viruses, virologists have opened a rational route to their control. I trust that the actual control of the viruses of higher organisms will not be far off.

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